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TITLE: Prevention and Treatment of Neurofibromatosis Type 1-Associated Malignant Peripheral Nerve Sheath Tumors

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14. ABSTRACT
The most common cause of death in Neurofibromatosis Type 1 (NF1) patients is malignant peripheral nerve sheath tumor (MPNST). MPNSTs are aggressive Schwann cell-derived neoplasms that typically arise from precursor lesions such as plexiform neurofibromas. Although gross total resection of MPNSTs is potentially curative, this occurs in only a small minority of cases. Radiotherapy and chemotherapy may inhibit local recurrence but have almost no effect on patient mortality. NF1 patients have an approximate 10% lifetime risk of developing an MPNST and this risk increases to approximately 30% in patients with plexiform neurofibromas. Thus, development of safe and effective MPNST preventative therapies could have an important impact on NF1 patient morbidity and mortality. In this grant, we are testing the hypothesis that chronic administration of agents that promote apoptosis and/or inhibit pro-survival autophagy will inhibit MPNST formation and progression in transgenic mouse models of MPNST. Specifically, we are examining the mechanisms of action and *in vivo* utility of two classes of drugs, BH3 mimetics and lysosomotropic agents, on MPNSTs. The drugs that we are testing are approved for human use and could be rapidly advanced into human MPNST clinical trials if our pre-clinical testing yields positive results.

15. SUBJECT TERMS
Apoptosis; autophagy; lysosomotropic agents; Bcl2 family members

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Title of Grant: Prevention and Treatment of Neurofibromatosis Type 1- Associated Malignant Peripheral Nerve Sheath Tumors

Award #: W81XWH-14-1-0073
Principal Investigator: Kevin A. Roth, MD, PhD
Annual Report: 04/01/2015– 03/31/2016

1. Introduction

Neurofibromatosis type 1 (NF1) has a frequency of approximately one in 3,000 humans and decreases life expectancy by ten to twenty years. Malignant peripheral nerve sheath tumors (MPNSTs) are the leading cause of death in NF1 patients and typically arise from NF1-associated precursor lesions. NF1 patients have an approximate 10% lifetime risk of developing a MPNST and this risk may be as high as 30% in NF1 patients with symptomatic plexiform neurofibromas. MPNSTs afflict NF1 patients in the prime of their lives, median age at diagnosis being approximately 40 years, and have a poor prognosis with median disease specific survival of approximately five to eight years. Gross total surgical resection is the only curative therapy and is unobtainable in the vast majority of patients. Radiotherapy and chemotherapy have proven largely ineffective in extending MPNST patient survival. Tumor formation and malignant progression are both dependent on the ability of tumor cells to evade normal cell death inducing stimuli. Numerous studies have shown that overexpression of anti-apoptotic Bcl-2 family members such as Bcl-2, Bcl-XL and MCL-1 can decrease tumor cell sensitivity to both radiotherapy and chemotherapy. Small molecule inhibitors of anti-apoptotic Bcl-2 proteins which have a functional BH3 domain, so called "BH3 mimetics", can potentiate tumor cell sensitivity to standard chemotherapeutic agents. Similarly, cytoprotective autophagy is commonly increased in tumor cells permitting these cells to survive in nutrient poor and hypoxic conditions that would kill normal cells. Cytoprotective autophagy can be inhibited by lysosomotropic agents such as chloroquine (CQ) which inhibit lysosome degradation of autophagic vacuoles and their contents. To date, no studies of combined BH3 mimetic and lysosomotropic agents have examined their potential utility as MPNST chemopreventive agents in either animal models or in NF1 patients.

2. Keywords

Apoptosis
Autophagy
Lysosomotropic agents
Bcl2 family members

3. Accomplishments

What were the major goals of the project?

1. To determine therapeutic effects of BH3 mimetics and lysosomotropic agents to inhibit Schwann cell hyperproliferation, MPNST formation and progression in transgenic mouse models.
2. To determine the effects and mechanisms of action of BH3 mimetics and lysosomotropic agents, alone and in combination, on NF1 patient-derived MPNST cell lines *in vitro*.

What was accomplished under these goals?

Since submission of our first year (04/01/2014 – 03/31/2015) annual progress report, we continued to make progress on the proposed studies described in that report. During the first five months of the second year of the award (04/01/2015 – 08/31/2015) we performed several large in vivo experiments and pursued multiple

studies on the effects of BH3 mimetics and lysosomotropic agents on malignant peripheral nerve sheath tumor (MPNST) cells in vitro. Progress on the specific experiments is listed below.
Effect of BH3 mimetics and lysosomotropic agents on Schwann cell proliferation in the sciatic nerves of young adult GGF beta 3 transgenic mice. We completed the initial study of the in vivo effects of two lysosomotropic agents (chloroquine; CQ and quinacrine; QA) and two BH3 mimetics (AT101 and ABT737) on Schwann cell hyperplasia in the transgenic mouse model. We found that the two BH3 mimetics at the doses tested had no effect on Schwann cell hyperplasia; however, both CQ and QA decreased Schwann cell proliferation in the experiment (mean BrdU labeling +/- SEM; Control: 733+/-231; CQ: 571+/-81; and QA: 441+/-78). Given these results, we focused on QA and performed a replicate in vivo study using two additional doses of QA. The injections were completed, tissue harvested and processed, and some of the sections were stained prior to Dr. Roth's departure. However, additional staining, counting, and analysis is required to complete the study.
Determine if BH3 mimetics and lysosomotropic agents inhibit the occurrence of MPNST in transgenic mouse models of MPNST. Over the first five months of year two of our award we completed the initial chronic in vivo study of the effects of CQ and AT101, alone and in combination, on MPNST tumor incidence and survival in the GGF beta 3 cross p53 +/- mouse model of MPNST. This large study involved multiple weekly injections to over 100 mice and a detailed histopathological analysis of the nervous system of all animals. Our analysis is not complete but we have some interesting initial observations that will inform the next study. First, chronic CQ by itself prolonged median survival from approximately 210 days to approximately 250 days but did not appear to influence the incidence of NF1 related tumors in the transgenic mice. Second, the BH3 mimetic AT101 did not increase median survival but did appear to decrease the incidence of MPNSTs. Third, the combination of CQ and AT101 did not appreciably increase the effects of the two agents alone. Finally, we are now in the process of performing detailed histopathological and statistical analyses on our findings prior to performing a replicate experiment after our mouse colony and grant are transferred to Columbia.
Define the molecular mechanism by which BH3 mimetics and lysosomotropic agents lead to MPNST cell death. The in vitro studies performed between April and August 2015 focused on the autophagy pathway as proposed in our original application and first year annual progress report. We focused extensively on our unexpected observation made in year one that BH3 mimetics dramatically suppress CXCL12 expression in MPNST cells. We are exploring the possible mechanisms by which this occurs and have expanded our analysis to include several MPNST cell lines and multiple BH3 mimetics to determine the general significance of this observation. Given that the CXCL12/CXCR4 autocrine growth axis has been implicated in NF1-associated MPNST formation and progression, these studies are particularly exciting and will become a major focus of our future studies.

Key Research Accomplishments

- Discovered that lysosomotropic agents chloroquine (CQ) and quinacrine (QA) moderately suppressed Schwann cell hyperproliferation in the sciatic nerves of young adult GGF beta 3 transgenic mice.
- We found that CQ extended median survial and AT101 decreased tumor incidence in a transgenic mouse model of MPNST.
- We determined that BH3 mimetics suppress CXCL12 expression in MPNST cells in vitro.

What opportunities for training and professional development has the project provided?
Dr. Roth met with colleagues at the recent Experimental Biology 2016 Meeting in San Diego, CA and discussed this work.

How were the results disseminated to communities of interest?
Dr. Roth presented lectures on this work at several institutions and at national meetings.

What do you plan to do during the next reporting period to accomplish the goals?
We will proceed with the previously proposed *in vivo* studies and expand our *in vitro* studies to include further evaluation of the CXCL12/CXCR4 pathway in MPNST progression.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?
BH3 mimetics and lysosomotropic agents are potentially useful new compounds for inhibiting MPNST formation and progression in NF1 patients but they require additional testing in animal models and further definition of their molecular mechanisms of action on MPNST cells. We have laid a solid foundation for these additional studies and we will proceed with the previously proposed *in vitro* and *in vivo* experiments as well as further investigating our novel observation that BH3 mimetics suppress CXCL12 expression in MPNST cell lines.

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

5. Changes/Problems

In year two, Dr. Roth moved from UAB to Columbia University and the grant was just recently transferred. Thus, there were some delays in the progress of the grant. Dr. Roth's laboratory is now established and the proposed experiments can now proceed as previously described.

6. Products

Publications, conference papers, and presentations

Journal publications
We are preparing manuscripts describing our findings but require additional time to replicate and extend key observations prior to submission.

Books or other non-periodical, one-time publications
Nothing to Report

Other publications, conference papers, and presentations
Nothing to Report

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Kevin A. Roth, MD, PhD – No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report

What other organizations were involved as partners?
Nothing to Report

8. Special Reporting Requirements

None

9. Appendices

None